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A NEW SIMPLE METHOD FOR DETERMINING VIABILITY OF YEAST CELLS VIA DYE EXCLUSION ASSAY

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ABSTRACT

Aim: This article describes a novel, easy-to-use technique for using the dye exclusion experiment to assess the vitality of yeast cells. **Methods:** Yeast cells were propagated and suspended in a solution of trypan blue and phosphate buffered saline. After that, the suspension was put into the Neubauer chamber for analysis. **Results:** The living cells showed no color; that is, they repelled the dye, whereas the dead cells looked blue, since the cell membrane of dead cells allowed the entry of the dye. The viable (live) cells present in a suspension can be quantified following an exclusion test employed in this article. The outcome is based on the fact that intact cell membranes in living cells prevent the entry of certain dyes, such trypan blue. **Conclusion:** In this paper, we report a simple trypan blue dye-exclusion method to determine the viability of yeast cells.

KEYWORDS: yeast, dye exclusion assay, trypan blue, viability test.

INTRODUCTION

Cell viability test refers to the number of living cells in a sample after certain period of time within a specified condition.[1] The specified conditions include reaction of the cell to external stimuli, chemicals, or medical treatments.[2]

Cell viability assays are increasingly being used in a wide range of sectors. There various cell viability tests including dye exclusion tests, colorimetric tests, flow cytometric, fluorometric and luminometric tests etc.[3] Dye exclusion assays include tests such as erythrosine B stain, congo red, eosin, and trypan blue. We provide a simple method based on the trypan blue dye exclusion experiment[1] for assessing the viability of yeast cells. The trypan blue assay is commonly utilized to determine changes in viable cell number generated by a medicine, toxin or any other desired or undesired substance. The trypan blue stain infiltration assay was initially established in 1975 to detect viable cell count.[4] Various other workers such as Strober (2019)[5] and Kucsera (2000)[6] have devised methods for dye exclusion assay.

MATERIALS AND METHODS

1g of commercially available dry yeast powder was added to 250 ml of autoclaved liquid broth with 1.25 g of peptone and 3 g of dextrose, yeast was incubated overnight at room temperature on a mechanical shaker (set to rotate between 120 and 125 rpm).

A 1.5 ml Eppendorf tube filled with 1000 ul of phosphate buffered saline, 150 ul of 0.4% trypan blue dye, and 50 ul of the aforementioned stock culture of yeast was used to create a cell suspension. PBS has a variety of applications; generally speaking, it is used to prepare cell suspensions since it is isotonic and non-toxic to most cells. Three minutes later, the suspension was placed into a Neubauer chamber for examination.

Following is the formula devised by us for calculating the cell viability

$$v = \frac{-\left(\left(\left(\frac{n_d}{n_l}\right) \times 100\right) - 100\right)}{100}$$

In above formula
v = cell viability
n_d= number of dead cells observed
n_l = number of live cells observed.

RESULTS AND DISCUSSION

Table 1 shows the sample findings. A cell viability value of 1 indicates that no dead cells were seen; a value less than 1 but greater than 0 indicates that the number

(percentage) of dead cells observed is less than the number (percentage) of living cells observed; and a value 0 indicates that an equal number (percentage) of dead and live cells were detected. A value <0 indicates that the number (percentage) of dead cells exceeds the number (percentage) of live cells observed. Thus, if the cell viability of any two samples A and B is 0.5 and 0.3, respectively, then the cells of sample A are more viable than the cells of sample B for the given set of circumstances.

Table 1: Table showing examples of observations and observed cell viability.

Sl no.	Number of dead cells (n_d)	number of live cells (n_l)	cell viability (v)	Total cell counted	percent of dead cell	percent of live cell
	0	100	1.00	100	0	100
1	15	85	0.82	100	15	85
2	23	77	0.70	100	23	77
3	33	67	0.51	100	33	67
4	40	60	0.33	100	40	60
5	50	50	0.00	100	50	50
6	60	40	-0.50	100	60	40
7	70	30	-1.33	100	70	30
8	80	20	-3.00	100	80	20
9	90	10	-8.00	100	90	10

Following the preparation of the cell suspension in PBS and trypan blue, Figure 1 displays a 400 x light microscopy microphotograph of a slide, and Figure 2 displays a 400 x light microscopy microphotograph of a slide taken three minutes later. Figure 2 depicts deep blue-colored dead yeast cells that have been dyed; living cells are not stained. The amount of live cells in the cell solution is counted considering the fact that live cells, because of their undamaged cell membranes, prevent the infiltration of dyes such as trypan blue.[7]

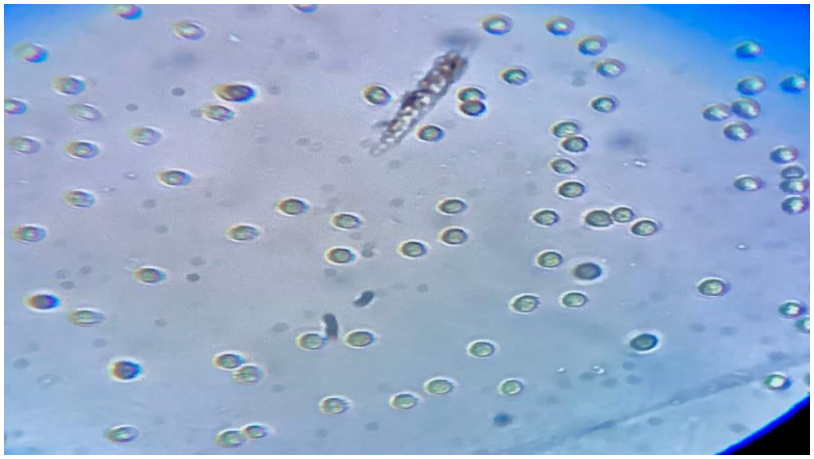

Fig. 1: Observation (400 x) immediately following cell suspension preparation using PBS and Trypan blue dye.

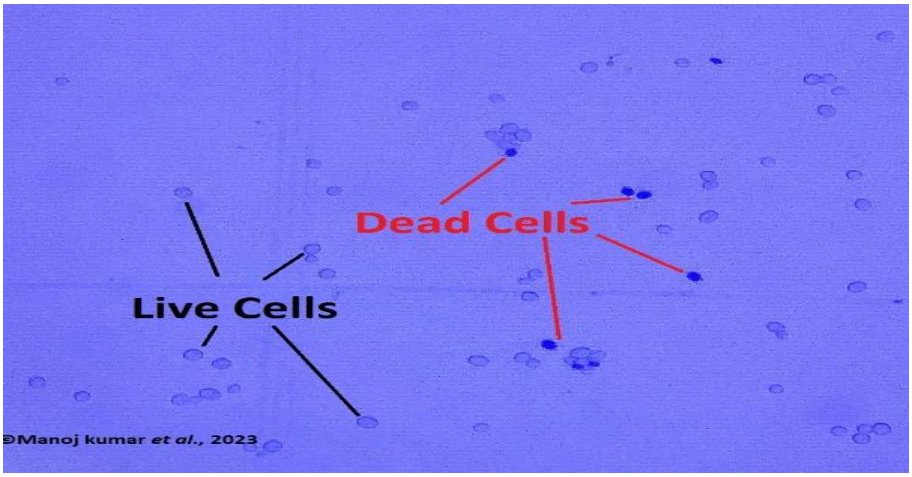

Fig. 2: displaying living cells (400x) that are not stained and dead cells that have been stained dark blue after three minutes of cell suspension preparation in PBS with Trypan blue dye.

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